REMARKS

In the Office Action dated March 30, 2011, claims 45, 46, 50-54, 60-61, 63-65, 68-71, 87-89, 91 and 133 were pending, with claims 63 and 91 withdrawn from further consideration. Claims 46, 50-54, 60-61, 64-65, 68-71, 87-89 and 133 were rejected under 35 USC §112, second paragraph, as allegedly indefinite. Claims 45-46, 50-54, 60, 64-65, 68-71, and 87-89 were rejected under 35 USC §103(a) based on the combination of Amit et al. (*Developmental Biology* 227: 271-278, 2000) ("Amit"), Mummery et al. (*Differentiation* 46: 51-60, 1991) ("Mummery"), Eiges et al. (*Current Biology* 11: 514-518, 2001), and Klug et al. (*J. Clin Invest* 98(1): 216-224, 1996).

Substance of Telephone Interview

A telephone interview was conducted among the undersigned attorney, Examiner Sgagias and Primary Examiner Ton on April 12, 2011. The principal references, Amit and Mummery, were discussed.

During the interview, Examiner Ton indicated that Amit was cited as a reference to hES cells, and that the skilled artisan would have considered it to be obvious to substitute the mouse EC cells of Mummery with hES cells given an understanding in the art that both cells can give rise to many differentiated cell types. The undersigned attorney disagreed and directed the Examiners' attentions to Applicant's previous submissions in respect to the distinctions between the EC cells and ES cells, and between mouse and human cells, including the Responses filed on October 26, 2009 and on July 16, 2010. These arguments had not been duly considered by the Office. Examiner Ton encouraged Applicant to reassert these arguments, and to identify any difference in the co-culture/differentiation conditions between the mouse EC cells of Mummery and the human ES cells of this invention. Examiner Ton also indicated that if Applicant's argument is directed towards lack of predictability and reasonable expectation of success in view of the art, the claims should reflect the scope of success demonstrated of record.

Applicant, through the undersigned, wishes to thank the Examiners for the helpful discussion of the case during the interview.

Claim amendments

Claims 51, 52 and 133 have been amended to delete the term "visceral endoderm-like", without prejudice or disclaimer. Claims 53 and 54 have been cancelled without prejudice or disclaimer. New dependent claims 134 and 135 are added herein, which define the embryonic cell to be END-2 cells, used in the co-culture with the hES cells. Support for claims 134-135 is found throughout the specification, e.g., page 11, lines 1-5 of the specification. No new matter is introduced.

Claim Rejections 35 USC § 112

The Examiner has rejected claims 46, 50-54, 60-61, 64-65, 68-71, 87-89 and 133 as allegedly indefinite.

a) The term "-like" in claim 51 and 133

Claim 51 and 133 stand rejected under 35 USC § 112 because the Examiner considers the term "-like" does not distinctly claim the subject matter and does not clearly describe the criteria necessary to identify the properties of the cells.

Without accepting the correctness of the Examiner's objections and comments, and to expedite prosecution of this application, the term "visceral endoderm-like" has been deleted from claims 51, 52 and 133. Claims 53 and 54 have been cancelled, without prejudice or disclaimer.

In view of the amendments presented herein, the rejection of the claims for reciting the term "-like" is overcome.

b) Claim 69

The Examiner rejectes claim 69 and contends that there is no antecedence to "the stem cells". Claim 69 has been amended to read "the stem cell" (singular), which obviates the rejection.

Claim Rejections 35 USC § 103

The Examiner has rejected claims 45-46, 50-54, 60, 64-65, 68-71, and 87-89 as allegedly unpatentable over Amit (2000) in view of Mummery (1991); Eiges (2001) and Klug (1996).

Amit (2000) in view of Mummery (1991)

Amit discloses culturing human embryonic stem cells (hES) on irradiated mouse embryonic fibroblasts (MEFs); however, the use of MEFs is taught by Amit to maintain the hES cells in an *undifferentiated* state, which is not relevant to the use of embryonic cell layers for the differentiation of human ES cells, as presently claimed.

Examiner Ton indicated during the telephone interview that Amit was merely cited as a reference to hES cells, and that the skilled artisan would have considered it to be obvious to substitute the mouse EC cells of Mummery with hES cells given an understanding in the art that both cells can give rise to many differentiated cell types.

Applicant respectfully disagrees. Applicant previously submitted the distinctions between the EC cells and ES cells, and between mouse and human cells, in Applicant's Response filed on October 26, 2009, for example. The Examiner considered the distinctions submitted by Applicant persuasive and withdrew the rejection based on van den Eijnden-van Raaij and Skerjanc, both of which are directed to mouse EC cells. Applicant presents these distinctions herein again for the Examiner's easy reference and in an interest to advance prosecution.

a) Distinctions - EC cells v. ES cells, and mouse v. human cells

Mouse EC cells are derived from malignant teratocarcinomas. They are generally karyotypically abnormal (aneuploid); in other words, they do not carry a normal complement of chromosomes. Applicant directs the Examiner's attention to an abbreviated copy of *Appendix C, C-8, Stem Cells: Scientific progress and future research directions – NIH publication June 2001 http:\\stemcells.nih.gov/info/scireport/appendixC.asp* (Exhibit 1 attached to Response filed October 26, 2009), which discusses the properties of EC cells and highlights the distinctions between human ES cells and EC cells (including mouse EC and human EC cells). The EC cells are adapted for tumor growth and when differentiated, show an inability to differentiate into well-recognized cell types. Even when transplanted, these cells retain the ability to form teratocarcinomas. EC cells are generally known as the *malignant* version of ES cells or cells of the inner cell mass (ICM), and generally do not differentiate significantly to a diversity of cell

types (see *Andrews, P.(2002) Phil. Trans R. Soc. Lond. B, 357, 405 – 417,* Exhibit 2 attached to Response filed on October 26, 2009).

Conversely, ES cells from different species are considered "genetically normal" as are the differentiated cell types that arise from them. ES cells are derived from the ICM of blastocyst stage embryos. Since they are karyotypically normal, when grown in immunodeficient mice, they form well-defined non-malignant teratomas with well-organized tissues representing all the three germ layers indicative of pluripotentiality (*Thomson et al 1998 referenced in Andrews, P (2002)*). In addition, when mouse ES cells are injected into a mouse blastocyst and the blastocyst is returned to the uterus, chimeric mice are formed in which most, if not all tissues (including the germ cells), can contain progeny of the donor mouse ES cells.

In contrast, mouse EC cells rarely contribute to many cells types in the chimera and additionally, rarely if ever contribute to the germ tissue as do the mouse ES cells. The ability of mouse ES cells to contribute so widely to different cell types either in teratomas or chimeras is a defining characteristic of mouse ES cells that demonstrates their true pluripotent nature (*Andrews, supra,* as Exhibit 2 attached to Response filed on October 26, 2009).

Notably many mouse and human EC cells have limited differentiation capacity or have completely lost their ability to differentiate and have become "nullipotent" (*Andrews et al., Biochemical Society Transactions 33, part 6, 1526-1530, 2005,* Exhibit 3 attached to Response filed on October 26, 2009, especially page 1527, right column, bottom paragraph). Therefore, even human EC cells do not behave in the same manner as human ES cells and have a very restrictive differentiation capacity.

Mouse ES and EC cells will generate muscle and mesodermal derivatives whereas human EC cells generally are not capable of mesodermal differentiation (*Draper, J.S. et al 2002 J. Anat. 200, 249 – 258*) (Exhibit 4 attached to Response filed on October 26, 2009). Human EC cells also commonly form the trophoblastic lineage while this does not occur with mouse EC cells (*Andrews et al. (2005), supra,* Exhibit 3 attached to Response filed on October 26, 2009).

The human EC cell line NTERA-2, one of the few human EC cell lines that is able to differentiate, is not capable of mesodermal differentiation and shows no evidence of cardiomyocyte differentiation (*Gokhale et al. 2000 Cell Growth Diff, 11, 157 – 162, referred to in Draper et al. (2002) (*Exhibit 4 attached to Response filed on October 26, 2009*) on page 256, left column, bottom of middle paragraph)*; whereas as shown in Mummery *et al.* (2002),

Circulation 107: 2733 – 2740 (Exhibit 5 attached to Response filed on October 26, 2009), hES cells are capable of being differentiated in a controlled and reproducible manner into cells of the mesodermal lineage and to form the cardiomyocytes (see abstract, for example).

The response of the mouse EC P19 cells to differentiation agents that enhance cardiomyocyte differentiation also differs from that of hES cells. The teaching of Skerjanc (cited by the Examiner in the Office Action dated June 15, 2009) highlights the required presence of 0.5-1% DMSO (dimethylsulfoxide) to induce cardiomyocyte formation from mouse EC P19 cells as aggregates in suspension culture. Importantly, DMSO has no effect on the differentiation of hES cells to the cardiomyocyte lineage (see *Xu et al., Circulation Research 2002; 91: 501-508*, Exhibit 6 attached to Response filed on October 26, 2009; see, e.g., the abstract). Therefore, it would not have been obvious that a differentiation inducing signal would have the same effect across both species and also cell type (e.g., mouse or human, EC versus ES cells). It is also notable that DMSO treatment of human EC cells fails to be an effective inducer of differentiation of human EC cells (see *Draper et al. (2002)* (Exhibit 4 attached to Response filed on October 26, 2009), page 254).

There are also prominent differences in the expression of cell surface markers, such as SSEA1, which is expressed on mouse EC and ES cells but not on human EC or ES cells. Human EC and ES cells also express glycolipids SSEA3 and SSEA4, proteoglycan antigens TRA 1-60, TRA 1-80 and GCTM-2 and protein antigens Thy1 and MHC class 1, all of which are not seen on mouse EC or ES cells (*Andrews et al. (2005), supra,* Exhibit 3 attached to Response filed on October 26, 2009).

Accordingly, the physical characteristics and the differentiation profiles of these cell types (mouse EC and human ES cells) are quite different. Observations made with one cell type cannot be extrapolated to another. In fact, the uncontrolled nature of mouse EC cells, particularly in their underlying karyotypic instability and response to differentiation agents make their predictability difficult in comparison to other karyotypically normal cell types such as human ES cells. Even human EC cell types are not able to respond to differentiating factors in a manner that is similar to hES cells, even though human EC cells can be differentiated to some lineages to a limited degree.

There is no correlation between the experiments performed in mouse EC cells with that performed in hES cells. When considering the abnormal nature of a mouse EC cell, one skilled

in the art would have had no reasonable expectation that conditions applied to mouse EC cells would apply to or operate with hES cells at all. This is because hES cells, although still regarded as a tissue culture artefact (*Zwaka T.P. and Thomson J.A 2005 Development, 132, 227 – 233)* (Exhibit 7 attached to Response filed on October 26, 2009), are far more representative of a normal pluripotent cell type than a mouse EC cell.

For all the above reasons, it is respectfully submitted that the generation of cardiomyocytes from hES cells, which has been demonstrated for the first time by the present invention, was entirely unexpected and therefore unobvious over the art which are directed to mouse EC cells.

(b) Further support to show differences between EC v ES and Human v Mouse

During the telephone interview, Examiner Ton asked that the co-culture/differentiation conditions be evaluated to identify differences in the conditions between the mouse EC cells of Mummery and the human ES cells of the invention.

Applicant directs the Examiner's attention to the experimental details provided in the specification, which show differences between EC and ES cells and between mouse and human cells cultured under the same conditions, which further supports the surprising nature of the results obtained when human ES cells are co-cultured with embryonic cells such as END-2 cells and are differentiated specifically to cardiomyocytes.

Specifically, both mouse EC cell line PC19 and a human EC cell line GCT27X were used in the experiments described in the examples of the present application, and their differences in both methods of culturing and response to the differentiation highlight the distinctions of these cell types.

In Example 4 of the specification (pages 26-32), four cell types were cultured as described. This example compares the responses of mouse ES (mES), human ES (hES), mouse EC (P19EC) (mEC) and human EC cells (GCT27X) (hEC).

All of the hEC, mES and hES cells required "feeders", whereas mEC (P19EC) were feeder <u>independent</u>. This emphasizes the difference between EC and ES cells for the mouse and human since both human EC and ES cell lines require feeder cells.

(i) Difference between mouse and human

On page 29, Example 4 (f)(i), mEC cells (feeder independent) cultured with END-2 cells aggregated spontaneously and after 7-10 days contained areas of beating muscle.

On page 30, Example 4 (f)(iii) hEC cells co-cultured with END-2 cells also aggregated but there was no evidence of beating muscle.

This shows that the mouse and human EC cells behave differently. Therefore a citation such as Mummery based on the mouse EC cells cannot be relied upon to predict the response of human cells or at least human EC cells.

(ii) Difference between EC and ES

On page 30 of the specification, Example 4 (f)(iv), hES cells were co-cultured with END-2 cells and beating muscle cells were evidenced between 12 and 21 days. This is in contrast to hEC cells in Example 4 (f)(iii) which did not respond to co-culture and provided no evidence of beating cells after three weeks in co-culture.

In Example 5, page 37, line 2, the specification states:

> "*the P19 EC assay system was preferred because these cells are feeder independent for undifferentiated growth.*"

A relevant reference is van den Eijnden-van Raaji *et al* 1991, previously cited by the examiner and withdrawn. It is stated on p164 of that paper in the material and methods section, that P19 cells are grown in "gelatinized flasks" with serum. That is, they do not require the use of a feeder cell layer to support their growth, in contrast to the hES cells used in the present application.

This further supports a difference between the EC and ES cells.

As noted above, the human EC cells do not result in beating cells when cultured with END2 cells. Thus, the skilled artisan would not have been motivated to even try co-culturing human ES cells with END2 cells, or at least would not have had a reasonable expectation of success that such co-culture would result in differentiation of human ES cells to cardiomyocytes.

Turning to Examiner Ton's argument that the skilled artisan would have considered it obvious to substitute the mouse EC cells of Mummery with hES cells, this argument does not

take into account the different feeder dependencies between the hES cells and mouse EC cells – the hES cells require feeders whereas mEC (PC19) cells did not require feeder support. Moreover, as shown above, EC cells behave differently from ES cells, and mouse EC cells behave differently from human EC cells.

Although a broad differentiation capacity is possible with both ES and EC cell types, it is not a predicable response to the same differentiation stimuli. For example, it is also noteworthy that hES cells maintained on the supporting mouse embryonic fibroblast (MEF) feeder cell layer were also unable to respond to END2 conditioned media (see p34, Line 33). As described in the current specification, the hES cells only responded after they were transferred from the MEF feeder layer to co-culture with the END2 cell line in the same serum containing hES media (hES complete medium).

Therefore there are two distinct effects of the "embryonic" feeder cells used:

1. Maintain pluripotency of hES cells. These are the MEF feeders which are used to maintain the cells in an undifferentiated state. The MEFs prevent cardiomyocyte differentiation with END2 conditioned media.
2. Induce cardiomyocyte differentiation from hES cells. The hES cells were transferred from the MEF feeder layer to co-culture with END2 to then be induced to differentiate to cardiomyocytes.

The difference between cell types is further exemplified in the current specification given the inability of a human EC cell line GCT27X ("with characteristics similar to human ES cells" noted on page 30, line 15) to differentiate to beating cells. It is noteworthy that a similar feeder independent EC cell line (the mouse P19 cells) does respond to both END2 co-culture and END2 conditioned media, further emphasizing the differences and unpredictability of the response of an ES or EC cell type that is dependent on both the source species as well as the cell type in their response to the same differentiation conditions.

In view of the foregoing, it is respectfully submitted that the Examiner's reliance on the combination of Amit and Mummery in establishing a *prima facie* obviousness case is flawed. One skilled in the art would have had no reasonable expectation that conditions applied to mouse EC cells would apply to or operate with hES cells at all.

Eiges et al. (2001) and Klug et al. (1996)

With respect to claim 70, the Examiner relies upon Eiges *et al.* and Klug *et al.* which teach the introduction of a gene into a human ES cell. Eiges and Klug merely describe methods of introducing the genes into a hES cell but provide no teaching to further differentiate the cell to a cardiomyocyte.

It is respectfully submitted that claim 70 is patentable by virtue of its dependency from claims 45-46, both of which are patentable for the reasons set forth above.

Examiner's closing remarks

- "*It would have been obvious to culture the hES cells of Amit in a system taught by Mummery to produce differentiated mesoderm cells or cardiomyocytes or vascular endothelial cells with a reasonable expectation of success.*"

As discussed above, Mummery's teaching is directed to EC cells which are distinct from ES cells. Furthermore, Mummery's EC cells are mouse EC cells which are feeder independent, in contrast to human ES cells which are feeder dependent. Moreover, EC cells do not produce beating areas when co-cultured with END-2 cells. Therefore, in light of these conflicting results, the distinct culture conditions and behaviour, those skilled in the art would not have been motivated to culture the hES cells with END-2 cells, simply because the co-culture may have worked with mouse EC cells. Certainly those skilled in the art would not have had a reasonable expectation of success in such an attempt.

- "*It would have been obvious to genetically modify the hES cells by introducing Rex 1-regulated gene markers (Rex1-EGFP) into human cells, allowing the determination of the differentiation status of the cells in culture such as taught by Eiges taken with Klug with a reasonable expectation of success.*"

It is respectfully submitted that the present invention is not directed to the genetic modification of hES cells *per se*, rather it is directed to the differentiation of a hES cell to a cardiomyocyte by co-culture with either END-2 cell or an embryonic visceral endoderm cell.

Any form of hES cells can be used, regardless of whether the hES cells are genetically modified or not. The genetic modification may be useful for identifying the differentiated hES cells that undergo differentiation as a consequence of the co-culture.

Summary

In view of the foregoing, it is respectfully submitted that it would not have been obvious to combine Amit with Mummery and to apply the mouse EC protocol of Mummery to human ES cells. Amit does not teach co-culture for the purposes of differentiation and hence does not teach toward Mummery. The mouse EC protocol of Mummery cannot be extrapolated to human ES cells, because the two cell types are distinct and the outcome would have been unpredictable as discussed above.

Applicant respectfully submits that the claimed invention is not obvious over the cited combination of art. Withdrawal of the obviousness rejection is respectfully requested.

Conclusion

It is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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